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CLINICOPATHOLOGICAL STUDY OF NON NEOPLASTIC LESIONS OF SKIN WITH SPECIAL EMPHASIS ON VESICULOBULLOUS LESIONS

Pathology

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ABSTRACT

Introduction: Dermatopathology is a unique branch of pathology, since it enables the clinician to directly view the patient's gross pathology and, as a result the success of specialty often depends much upon careful clinicopathological correlation as it does upon histological features particularly.

Aims and Objective: 1.To classify various non neoplastic lesions into categories that predicts clinically important attributes such as prognosis or response to therapy.2. Clinicopathological correlation to analyze the different nonneoplastic lesions of skin.

Materials and Methods: A hospital based prospective study was conducted from June 2008 to May 2010 on 205 patients attending the outpatient Department of Dermatology & Venereology and Pathology with various non neoplastic lesions of skin.Biopsy of each lesion was done with routine procedure and histopathology was performed according to the study protocol including special stains.

Results: Out of 205 patients clinicopathological correlation seen in 82.43% of the cases giving our significant p value of 2.2e-16 (which is much less then 0.0001).Vesiculobullous diseases found to be correlated clinicopathologically (77.14%) with p value of 0.000939 which is significant.Hundred percent correlations were seen in Photosensitivity Disorders, Metabolic Diseases of skin, Protozoan & Parasitic Diseases and Vascular diseases.

Conclusion: Our study revealed that Clinicopathological correlation is highly significant in dermatopathology ,making accurate diagnosis in majority of non neoplastic skin disorders thus guiding appropriate treatment of patients and in assessing prognosis.

KEYWORDS

INTRODUCTION :

The histopathologic diagnosis is used by clinicians to aid in the management of patients.The most accurate diagnosis is the one that most closely correlates with clinical outcome and helps to direct the most appropriate clinical intervention thus depicting a close relationship between diagnosis and prognostification.

Despite the incremental advances of molecular techniques in diagnosis,surprisingly morphology is still the basis of diagnosis for most neoplasms and many inflammatory dermatosis.[1]

Although histopathology remains the gold standard for most dermatologic diagnosis,it must be recognized that not all lesions are amelnable to definitive “specific” histologic diagnosis.Foucar (1995) has elegantly discussed the difficulties inherent in making a histologic diagnosis and the issues have also been well covered by Sackett et al (1985). [2,3]

A major source of difficulty in making an exact diagnosis in pathology,as in clinical medicine,is that the information required to make the diagnosis is frequently incomplete at some level,or at multiple levels.At the most fundamental level,there may simply be no extent standard for a particular diagnosis.

Thus it is obvious that histopathologist can give clinician a maximum amount of information only if every specimen submitted is accompanied by detailed clinical information including diffential diagnosis.

The histologic features of many inflammatory dematosis in particular are nonspecific or ,at best,only suggestive of a specific diagnosis.Even among the more readily characterized papulosquamous dermatosis ,such as psoriasis or lichen planus,the histologic picture is more typically “compatible with”rather than “diagnostic of” the clinical picture. Skin biopsy is an invaluable tool in the diagnostic armamentarium of a dermatologist. It not only helps in diagnosis in cases of dilemma but also provides an opportunity to find something unusual in routine practice. In the era of evidence-based medicine, consumer activism and litigations, skin biopsy helps in ensuring documentary evidence for the diagnosis made and the basis for the treatments started. Hence, it is important to have the basic knowledge of techniques of skin biopsy so as to derive the maximum from the procedure. [1]

The pathologist who wishes to interpret histopathological section of skin diseases must familiarize themself with clinical features of at least the common diseases. As the gross appearance of lesions and their distribution is an aid to him, like the naked eye appearance of organ or specimen in general histopathology, nothing on gross can be gained from skin biopsy specimen received which is in millimeters to centimeters.

The dermatologist also must be aware of potentials and limitations of skin biopsy and above all appreciate the fact that skin has a limited number of reaction patterns with which it can respond to various pathological stimuli. If they will appreciate this fact, then only they will understand why the clinically different lesions may show similar histopathologic pattern.

If significant help is to be obtained from skin biopsy then it should be accompanied with all clinical details, site of biopsy, duration of treatment, may be local, or systemic and clinical differential diagnosis. In the end skin biopsy is vital to the understanding of the pathological processes that underlie skin diseases. The dermatologist describes the gross pathology of skin diseases whereas histopathologist attempts to describe the microscopic pathology. Hence a correlation of both is essential to reach the diagnosis.

AIMS AND OBJECTIVES

1. To classify various non neoplastic lesions into categories that predicts clinically important attributes such as prognosis or response to therapy.
2. Clinicopathological correlation to analyze the different nonneoplastic lesions of skin.

MATERIAL AND METHODS

The study was conducted on 205 patients who attended the outpatient

Department of Dermatology & Venereology and Pathology, in NKP Salve Institute of Medical Sciences and Research Centre.during a period extending from June 2008 to May 2010.

Inclusion criteria
- All patients male, female of any age group presenting with lesions of skin clinically suspected non neoplastic.

Exclusion criteria
- Patients diagnosed as neoplastic disorder of skin.
- Patients with ruptured bullae.
- Patients with secondarily infected or heavily scratched areas.
- Patients with involuting lesions.
- Patients already on topical steroids or anti-inflammatory drugs for lesion as they suppress inflammatory response or encourage secondary invasion by bacteria or fungi.
- Patients with healed or healing lesions.

WITHDRAWAL CRITERIA
- Patient's refusal for any procedure to be carried out for research.

Method of collection of data
Pertinent clinical history like age, duration of the lesion, site of the lesion, significant family and personal history, history of associated diseases and any drug intake was taken and entered in the proforma. After detailed general and local examination, the site of the biopsy was selected. The selected patients consent was taken after explaining the details of the biopsy procedure. The biopsy is done on the lesion along with the surrounding area. The biopsy area is cleaned & painted with an antiseptic solution and adequate amount of local anaesthetic (2% lidocaine) is injected to the skin and subcutaneous tissue.

Biopsy Technique
A specimen obtained with a 4mm biopsy punch is adequate for histologic study. A 3 mm punch may be preferable for small lesions or biopsy from face for cosmetic reasons. After the skin specimen has been loosened with the biopsy punch instrument, it should be handled very gently and should not be grasped with forceps.

It should be gently squeezed out of its socket, or be carefully spread with the syringe needle that was used for the injection of local anaesthetic. The biopsy specimen taken should be placed in a fixative immediately on removal from the patient to prevent autolysis.

As fixative 10% formalin was used in almost all instances. The biopsy Specimen provided should include history of previous biopsies, adequate clinical history if any and any special requests if required. The histopathologist's ability to render an accurate diagnosis often depends on the available clinical information. Every specimen submitted to histologic diagnosis was accompanied with detailed clinical information, including differential diagnosis.

Gross examination of the skin biopsy
The three dimensional size and shape of the skin biopsy was assessed including the circular or elliptical shape of the biopsy.

The entire skin biopsy was submitted for routine processing and embedded in paraffin wax. 3-5mm thick paraffin sections of the skin biopsy were stained with haematoxylin and eosin.

HAEMATOXYLIN AND EOSIN STAINING
Procedure
1. Paraffin sections placed in xylol for 2 minutes
2. Transferred to absolute alcohol for 1 minute
3. Section drained and placed in 90% alcohol for 1 minute
4. Section transferred to haematoxylin for 10-40 minutes
5. Slides are transferred to slide washing tray for blueing for 10 minutes
6. Section dipped in acid alcohol, agitated for few second for differentiation.
7. Section transferred to slide washing tray for 3-4 min to differentiate eosin.
8. After draining, section transferred to 90% alcohol agitated for 10-15 seconds
9. Slides transferred to absolute alcohol agitated for 10-15 seconds.
10. Slides transferred to absolute I and then to absolute II for 30 seconds.
11. Sections transferred to Xylol I and Xylol II until completely clear
12. Sections mounted with DPX.

The sections were usually stained with H&E stain. If necessary the special stains used were :-
- Periodic acid Schiff (PAS)
- Fite Faraco
- Congo red

Statistical Analysis :-
Aim of the study was to determine the statistical significance of clinically correct diagnosis of patients in various disease categories. The correct diagnosis is the one, which is supported positively by histopathological findings. In this study, we define two possibilities, viz success and failure. A diagnosis is said to be a 'success' if both clinical and histopathological findings point towards same conclusion; and 'failure' if they contradict. Accordingly, we set up the hypothesis that chances of success and failure are the same i.e. 0.5 as against the alternative hypothesis that the chances of success are more than failure.

To test this hypothesis, we used Binomial Exact Test with 5% level of significance.

RESULTS
Number of cases Study included 205 patients between the age group ranging from 2-79 years with a mean age of 39 years.Maximum number of patients were seen in the age group 30-39 years which consisted of 25 patients (22.43%) with male predominating which can be attributed to more exposure of males to work.(FIG-1)

Figure-1: Age and Sex wise distribution of diseased patients.

Name of disease	Total No. of cases	Percentage
Genodermatoses	4	1.95
Non-infectious, Erythematous, papular and squamous diseases	64	31.21
Vascular diseases	2	0.98
Non-infectious vesiculobullous vesiculopustular diseases	35	17.07
Connective tissue disorders	15	7.32
Photo sensitivity disorders	1	0.49
Non-infectious granuloma	4	1.95
Metabolic diseases of skin	3	1.46
Inflammatory diseases of hair follicles, sweat gland and cartilage	2	0.98
Bacterial diseases	43	20.98
Protozoan and parasitic diseases	1	0.49
Diseases caused by viruses	8	3.9
Miscellaneous	23	11.21
Total	205	100 percent

Out of 205 cases maximum number of skin lesions was found of non infectious erythematous, papular and squamous diseases followed by bacterial diseases, non infectious vesicular and bullous diseases. (Table-1)

Table 2: Clinicopathological correlation of various non-neoplastic skin lesions

Name of Disease	No. of cases	Histopathologically correlated cases	Percentage
Genodermatoses	4	4	100
Non-infectious, Erythematous, papular and squamous diseases	68	62	91.17
Vascular diseases	2	2	100
Non infectious vesiculubullous vesiculupustular diseases	35	27	77.14
Connective tissue disorders	15	15	100
Photo sensitivity disorders	1	1	100
Non infectious granuloma	5	4	80
Metabolic diseases of skin	3	3	100

Inflammatory diseases if hair follicles, sweat gland and cartilage	3	2	66.66
Bacterial diseases	60	41	68.33
Protozoan and parasitic diseases	1	1	100
Diseases caused by viruses	8	7	87.50
Total	205	169 (82.43%)	

Above table shows that clinicopathological correlation of non neoplastic skin lesion was seen in 82.43% of cases.(Table-2)

Hundred percent correlation was seen in genodermatosis, photosensitivity disorders, metabolic diseases of skin, protozoan & parasitic diseases and vascular diseases.

Non Infectious, Erythematous, Papular and Squamous diseases showed significant correlation 91.17%.

Non infectious vesicular and bullous diseases also showed significant correlation 77.14%.

DISCUSSION

The present study was undertaken to determine the incidence of non neoplastic lesions of skin and their clinicopathological correlation with special emphasis on vesicobullous lesions.Total 205 cases of skin lesions were studied.

In our study, we found the highest number of cases of non-infectious erythematous, papular and squamous disorders (31.21%), followed by the bacterial diseases (20.98%) and then non-infectious vesiculobullous and vesiculopustular diseases (17.07%). Connective Tissue disorders constituted 7.32% of all lesions. In the field of Dermatopathology, often no diagnosis is made. The histopathology findings are suggestive of diagnosis or nonspecific changes.[1]

In our study 105 patients were male and 100 patients were female with maximum number of cases occurring in fourth decade (21.95%).

The highest number of cases (31.21%) in the Non-infectious Erythematous, Papular and Squamous disorders can be attributed to various factors like trauma, infection, metabolic causes, drugs and endocrinal provocative factors.

DISTRIBUTION BY HISTOPATHOLOGICAL PATTERN

GENODERMATOSES

In the present study four cases of the congenital skin diseases were reported.

DARIER's DISEASE

The Darier's disease, though genodermatoses, may arise late in life Glissen & Mobacken (1975) and may be localized.[4] The only case reported was a male, 34 years having symptoms since 15 days. On histopathology crops ronds and grains formation, suprabasal acantholysis was seen.(Figure-2).

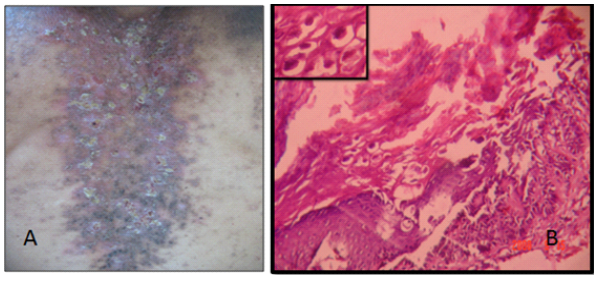

Figure-2.(A) Clinical photograph of Darier disease showing warty papules & plaques covered with greasy crust on sternal region . (B) Photomicrograph of Darier`s disease showing corps ronds, grains formation and suprabasal acantholysis(H&E x 100) Inset showing grains formations in horny layer(H&E x 400)

ACROKERATOSIS OF Hopf

Acrokeratosis verruciformis of Hopf is a rare autosomal dominant disorder appearing at birth or in early childhood, but the onset may be delayed upto 5th decade Ebling FJG (1987).[5] We reported one case of female, 47 years old having symptoms since 3 months. On histopathology considerable hyperkeratosis, increase in thickness of the granular layer, acanthosis and moderate papillomatosis was seen.(Figure-3)

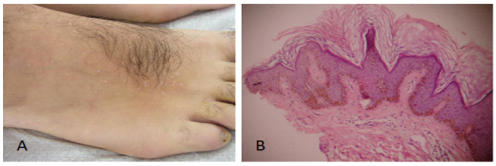

Figure-3.(A) Clinical photograph of Acrokeratosis verruciformis of Hopf showing skin coloured warty hyperkeratotic lesions.(B) Photomicrograph of Acrokeratosis verruciformis of Hopf showing marked papillomatosis, hyperkeratosis,increased granular layer (H&E x 100)

POROKERATOSIS

Porokeratosis is inherited in an autosomal dominant pattern. We report two cases one male and one female both in third decade. On histopathology hyperkeratotic ridge shows a keratin-filled invagination of the epidermis.

Non Infectious Erythematous Papular And Squamous Diseases

Among non infectious erythematous papular and squamous diseases we reported 5 cases (7.8%) of Erythema Dyschromicum Perstans,16 cases (24.96%) of Psoriasis,31 cases (48.43%) of Lichen planus and its variants,4 cases of Inflammatory linear verrucous epidermal nevus,2 cases of Pityriasis lichenoides chronica and 6 cases of Prurigo nodularis.

Erythema Dyschromicum Perstans was seen in third and fourth decade. On histopathology, epidermal atrophy, pigment incontinence in dermis and perivascular infiltrate was seen.

Maximum number of psoriasis cases was seen in 5th and 6th decade, out of which 15 were males and one female. We found a male preponderence in our study which is consistent with Grace D`Costa et al (2010), however Bell et al (1991) found a female preponderence and Fry (1988) found no sex prediliction.[6,7,8] Out of 16 cases one case was of pustular psoriasis in which spongiform pustule was seen. On histopathology elongated rete ridges, acanthosis, focal spongiosis and mounds of parakeratosis was seen in most of the cases.(Figure-4)

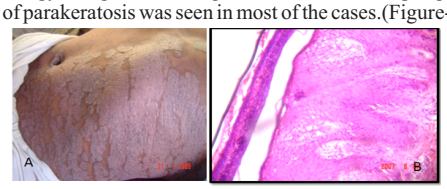

Figure 4(A) Clinical Photograph of Psoriasis showing variable sized lesions, sharply demarcated, dry, covered with layers of fine silvery scales. (B) Photomicrograph of Psoriasis showing moderate parakeratosis, hyperkeratosis, slight elongation of rete ridges.(H&E x 100).

Lichen planus showed little female preponderence in our study which is consistent with Grace D`Costa et al (2010).[6] On histopathology band like lymphocytic infiltrate in dermis, focal hyperkeratosis, irregular acanthosis was seen. In few cases Max-Joseph space was also seen. Civatte bodies were seen in only eleven cases (35%) where as Garg VK et al (2000) reported Civatte Bodies in 37% of cases.[9]

Inflammatory Linear Verrucous Epidermal Naevus is composed of erythematous, slightly verrucous, scaly papules arranged in one or several lines. On histopathology increased granular layer, hyperkeratosis, parakeratosis, slight papillomatosis and acanthosis was seen.[10](Figure-5)

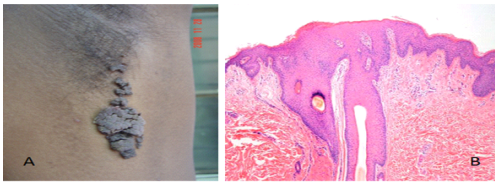

Figure-5.(A) Clinical photograph of Linear inflammatory verrucous epidermal nevus showing erythematous, slightly verrucous, scaly papules arranged in one line. (B) Photomicrograph of Linear inflammatory verrucous epidermal nevus showing hyperkeratosis with foci of parakeratosis, moderate acanthosis, elongation and thickening of the rete ridges (H&E x 100)

VASCULAR DISEASES

Sweet Syndrome

In our study we reported one case of female in 4th decade which is similar to Bharija C.S. et al (1995) who also reported 10 cases of sweet syndrome who were females ranging in age from 30–50 years.[11] On histopathology a dense perivascular infiltrate composed largely of neutrophils was seen throughout the papillary dermis as well as marked edema in upper dermis.

Leucocytoclastic vasculitis (LCV)

In our study we reported one case of female in 4th decade. Possible etiological agent of LCV was unknown and the lesions were on lower limbs. On histopathology neutrophils in vessel wall, fibrinoid necrosis and extravasation of red blood cells was seen. (Figure-6).

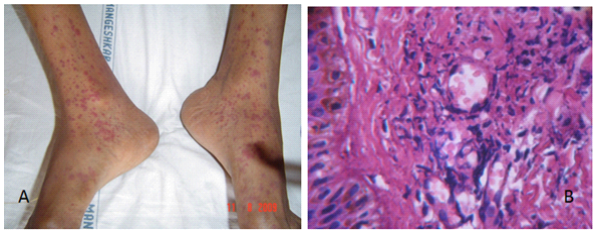

Figure-6.(A) Clinical photograph of Leucocytoclastic vasculitis showing bilateral macular erythematous cutaneous rash.(B) Photomicrograph of Leucocytoclastic vasculitis showing perivascular neutrophilic inflammatory infiltrate, karyorrhectic debris with extavasation of red blood cells(H&E x 400)

NON INFECTIOUS VESICULAR AND BULLOUS DISEASES:

The largest cases reported in the non infectious vesicular and bullous diseases were that of dermatitis 39.96% with slight male predominance. The cases ranged from first decade of life to seventh decade predominating in second and fourth decade.

Other lesions in this category included 13 cases (37.1%) of Pemphigus, out of which 11 cases (31.4%) were of pemphigus vulgaris, one each of Pemphigus Foliaceous and Pemphigus Vegetans (2.85% each). Maximum number of cases belongs to 40-69 years of age whereas M M Huda et al (2001) reported cases between 31-50 years of age.[12] The histopathologic findings of pemphigus vulgaris were intraepidermal suprabasilar bulla in 9 cases (81.8%) and mid epidermal bulla in 2 cases (18.18%) with desquamated cells within it and infiltrate in dermis. This is similar to study of Arya et al (1999). [13]

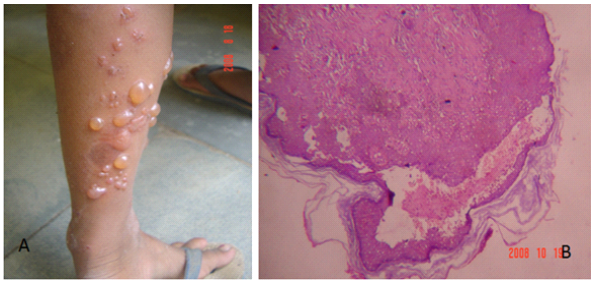

Figure-7.(A) Clinical photograph of Pemphigus vulgaris showing multiple fluid filled bullae on leg.(B) Photomicrograph of Pemphigus vulgaris showing intraepidermal bulla containing fluid and acantholytic cells (H&E x 400)

Bullous Pemphigoid and Erythema Multiforme constituted 4 cases each (11.4%).

BACTERIAL DISEASES

Among the bacterial disease the two diseases common in India were also found in the present study with highest number of cases which included 4 cases (9.3%) of tuberculosis of skin and 38 cases (88.27%) of leprosy. Lupus vulgaris is commonest type of cutaneous TB in our study as observed in study by Pandhi RK et al (1977.[14] Two cases (50%) had lesion on face, similar to Bhambani et al (1991) who reported lesions on face in 40% of cases.[15] On histopathology tuberculoid granulomas composed of epithelioid cells and Langhan's giant cells was seen in lupus vulgaris.

Among leprosy, number of cases of borderline tuberculoid leprosy was highest, 22 cases (51.1%), tuberculoid leprosy 13 cases (30.2%), lepromatous leprosy 3 cases (6.97%). Maximum number of patients was males with 68.42% females 31.5%. This is similar to Moorthy B.N. et al (2001) who reported leprosy in 65.05% males and 34.95% females.[16]

DISEASES CAUSED BY VIRSUSES

In our study 2 cases of molluscum contagiosum, 4 cases of verruca plana and 2 cases of verruca vulgaris were reported. Mahajan BB et al (2003) reported that Molluscum contagiosum is one of the commonest cutaneous viral infections especially in school going children.[17] (Figure-8).

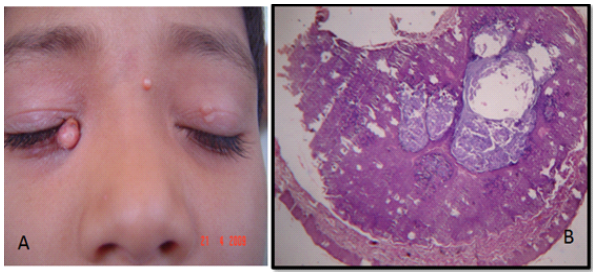

Figure-8.(A) Clinical photograph of Molluscum Contagiosum showing skin coloured, delled, dome-shaped papules. (B) Photomicrograph of Molluscum contagiosum showing numerous intracytoplasmic inclusion bodies, so-called molluscum bodies discharging into dermis (H&E x 100)

On histopathology, verruca plana revealed hyperkeratosis, slight elongation of rete ridges, with no papillomatosis and no areas of parakeratosis. These findings are similar to which reported by Halder et al (2009) in their study. [18]

METABOLIC DISEASES

Three cases of Lichen Amyloidosis were reported. On histopathology amyloid globules were seen in papillary dermis in Lichen Amyloidosis. One case of lichen amylodosis was positive for Congo red stain.

CONNECTIVE TISSUE DISEASES

Among the connective tissue diseases we reported three cases (20%) of scleroderma and all were females. Ahathya RS et al (2007) also reported that females are affected seven times more than males. [19] On histopathology increased collagen in dermis with atrophy of sweat glands was seen.

Other connective diseases reported were 6 cases (40.00%) of Morphea, 2 cases (13.30%) of Discoid Lupus Erythematosus and 4 cases of Systemic Lupus Erythematous.

PHOTOSENSITIVITY DISORDERS

Polymorphic Light Eruption

One case of PMLE was reported who was a fair skinned individual and had external aspect of arms and forearms involved. On histopathology normal epidermis, mild spongiosis and mild perivascular and periadnexal, lymphohistiocytic inflammatory cell infiltrate was seen.

CLINICAL PATTERN OF SKIN LESIONS

Maximum number of skin lesions were of macular type (90 cases) followed by papular (65 cases), then vesicullobullous (35 cases).

CLINICOPATHOLOGICAL CORRELATION

In the present study clinicopathological correlation was observed in 169 cases (82.43%). A quite significant number of cases showed nonspecific changes on histopathology, clinicopathological correlation could not be obtained in such cases.

Hundred percent correlation seen in Genodermatosis, Photosensitivity Disorders, Metabolic Diseases of skin, Protozoan & Parasitic Diseases and Vascular diseases.

Non- Infectious, Erythematous, Papular and Squamous diseases showed 62 out of 68 cases corelated clinicopathologically (91.17%) which is similar to Grace D'Costa et al (2010) who got positive corelation in 97.52% of cases. In our study leprosy showed 84.44% corelation, whereas in study done by Moorthy BN et al (2001), there was aggrement in 62.63% of cases. Tuberculosis of the skin and papulonecrotic tuberculid showed 100 percent correlation.[6,16]

In diseases caused by viruses correlation was seen in 87.5% of cases. Non infectious vesicular and Bullous diseases also showed significant correlation 77.14%. Tzanck test was done in 21 out of 35 vesiculo bullous diseases and 16 out of 21 cases correlated with histologic diagnosis. Sensitivity of test being 80% which is almost similar to Fauziya Sabir et al (2010) who got 96% sensitivity.[20]

Tzanck test performed in all the patients of Pemphigus Vulgaris showed positive results with acantholytic ballooning cells which corresponds to Ratan Singh et al (1973).[21]

The statistical analysis was carried out for all vesiculobullous diseases and the statistical significance of clinically correct diagnoses was evaluated. Out of 35 cases, in 27 cases, the histopathological findings supported the clinical opinion. To test whether this occurence is significant at 5% level, Binomial exact test was applied. The test resulted into a *p*-value of 0.000939 which is less than 0.001, implying that the chances of correct clinical diagnosis are significantly higher than that of failures.

Aim of the study was to determine correlation between clinical and histopathological findings to form correct diagnosis. The correct diagnosis is the one, which is supported positively by histopathological findings. In this study, we define two possibilities, viz success and failure. A diagnosis is said to be a 'success' if both clinical and histopathological findings point towards same conclusion; and 'failure' if they contradict. Accordingly, we set up the hypothesis that chances of success and failure are the same i.e. 0.5 as against the alternative hypothesis that the chances of success are more than failure.

To test this hypothesis, we used Binomial Exact Test with 5% level of significance. The test resulted into a p-value of 2.2e-16, which is much less than 0.0001, thereby indicating that the chances of success are significantly higher than that of failure.

The power of the study was also evaluated using proportion of success under null hypothesis p_0 as 0.5, proportion under alternative p_1 as 0.8, significance level of 5% and the sample size of 205. The resulting value was 0.999, indicating the sample of 205 has 99% probability of giving a significant ($p<0.05$) exact binomial test.

CONCLUSION

Clinicopathologic correlation is the crux and beauty of dermatopathology; no other branch of medicine has so many different names for the appearance of an organ and in many cases, each of the peculiar names has a histological correlate. On the other hand, the skin can only react in so many ways, so that some patterns such as “superficial lymphocytic perivascular infiltrate with sparse admixture of eosinophils” can be associated with many different diagnoses ranging from viral exanthema to drug reaction to bullous pemphigoid. The digital age has made it convenient to provide the dermatopathologist not only with a detailed clinical history but also with clinical pictures (e.g., digital images), both of which increase the chances of the clinician receiving a helpful diagnosis.

Last but not least when histological picture in not diagnostic, clinicopathological correlation frequently makes diagnosis possible.

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